

the phrase "the wild-type gene is inactivated in the cell". Support can be found on p. 9 lines 8-12 and 17-19 of the specification, which describes the Environmentally Limited Viability System ("ELVS") as combining specific regulation with essential gene *and/or* lethal genes to limit the viability of a microorganism to a permissive environment. Thus, limited viability of a microorganism to an environment can be achieved by simply regulating the essential gene with or without the lethal gene. New claims 66-85 have been added to clarify that, in these particular embodiments, the essential gene native to the host cell can be inactivated, and the claimed invention can still operate as intended because an active copy of said essential gene is expressed in a permissive environment and not expressed in a non-permissive environment. Support can be found in the specification, for example, on p. 6, lines 5 - 11, Figure 4, and on p. 46, lines 10-22, to p. 50 lines 24.

#### REJECTION UNDER NON-STATUTORY DOCTRINE OF OBVIOUSNESS-TYPE DOUBLE PATENTING

Claims 1-4, 8-14, 16, 20, 23-24, 27-32, 35, 37 and 41-44 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over U.S. Patent Application Ser. No. 08/761,769. As there are no allowable claims in the instant application at this time, Applicants wish to defer responding to this rejection until such time as there are allowable claims.

#### REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 1-4, 8-14, 16, 20, 23-24, 27-29, 30-32, 37 and 41-44 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Office alleges that the phrase "wild type gene is inactivated in the cell" render the essential gene inactivated by any means and at any time, such that the essential gene could not be expressed even in a permissive environment.

Applicants respectfully submit that the phrase "wild type gene is inactivated in the cell" has been deleted from claims 1, 27, 30 and is not recited in new claims 46-65. However, as recited in new claims 66 - 85, the essential gene can be inactivated, and the claimed invention can still operate as intended. That is, the essential gene is expressed in the permissive environment and is not expressed in the non-permissive environment. In that embodiment, an essential gene native to the host cell is inactivated and thus, not expressed in any environment.

Inactivation can be achieved by any means known by those skilled in the art or taught in the specification. For example, the specification (p. 30, lines 13-16) teaches a deletion of the *asd* gene to inactivate it. Alternatively, a skilled artisan can use site-directed mutagenesis to inactivate the essential gene. An active *copy* of said essential gene is introduced into said host cell, and that copy of the essential gene is expressed in a permissive environment, but not expressed in a non-permissive environment.

In short, the Environmentally Limited Viability System in the claimed microbial cell comprises at least an essential gene that is specifically regulated to turn on and off in the permissive and non-permissive environments, respectively. In another embodiment, the system further comprises at least one lethal gene which is expressed in a non-permissive environment and not expressed in the permissive environment. Cell death in a non-permissive environment, therefore, can be achieved by the expression of a lethal gene and the nonexpression of the essential gene. In still a further embodiment, an essential gene, native to the host cell, is inactivated and an active copy of said essential gene is introduced into the host cell and is expressed in the permissive environment and not expressed in a non-permissive environment. In essence, each embodiment requires the regulation of an essential gene in the permissive and non-permissive environments.

Applicants, therefore, submit that the claims, as amended and introduced, are clear and not indefinite and respectfully request the Office withdraw its rejection under 35 U.S.C. § 112, second paragraph.

#### REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 1-4, 8-14, 16, 20, 23-24, 27-29, 30-32, 37 and 41-44 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. Specifically, the Office alleges that all of the claims recite dead cells because the essential gene is inactivated under any conditions, permissive or nonpermissive.

Applicants respectfully submit that the amended claims 1, 27, and 30 and new claims 46-65 do not recite dead cells as they do recite expression of an essential gene for viability. First, the claims no longer recite "wherein the wild type gene is inactivated in the cell". Second, as discussed above, if an essential gene native to the host cell is inactivated, as in claims 66-85, then an active copy of said essential gene is introduced into said cell, allowing said

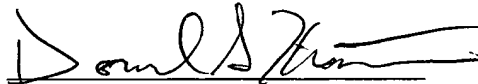
active copy of the essential gene to be expressed in a permissive environment, but not expressed in a nonpermissive environment.

Applicants respectfully request reconsideration and withdrawal of the Office's rejection under 35 U.S.C. § 112, first paragraph.

### CONCLUSION

In light of the above amendments and remarks, Applicants believe all of the Office's rejections have been overcome or obviated and that the claims are in form for allowance, and respectfully request that such allowance be granted.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Daniel S. Kasten", written over a horizontal line.

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AMENDED AND ADDED CLAIMS

1. (Four times amended) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

27. (Four times amended) A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment.

30. (Five times amended) A method of inducing immunoprotection in a warm-blooded animal comprising administering to the animal a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said [the] essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and wherein the cell is a member of the *Enterobacteriaceae*.

46. (New) The cell of claim 1 further comprising a lethal gene, wherein expression of the lethal gene is lethal to the cell and wherein the lethal gene is expressed when

the cell is in the non-permissive environment but not when the cell is in the permissive environment.

47. (New) The cell of claim 46 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

48. (New) The cell of claim 46 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of the *Enterobacteriaceae*.

49. (New) The cell of claim 46 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

50. (New) The cell of claim 49 wherein the vector has two lethal genes.

51. (New) The cell of claim 46 wherein expression of the essential gene is regulated by the expression product of a regulatory gene.

52. (New) The cell of claim 51 wherein the expression product of the regulatory gene inhibits expression of the essential gene and said expression product is expressed or active only in the non-permissive environment.

53. (New) The cell of claim 49 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

54. (New) The cell of claim 46 further comprising an expression gene wherein the expression gene encodes a desired expression product.

55. (New) The cell of claim 46 [for use as a vaccine], wherein the cell is viable when in an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C, wherein the cell is a member of *Enterobacteriaceae*.

56. (New) The cell of claim 55 further comprising an expression gene wherein the expression gene encodes a desired expression product.

57. (New) The method of claim 27 further comprising stably introducing into a cell a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

58. (New) The method of claim 57 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

59. (New) The method of claim 57 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of *Enterobacteriaceae*.

60. (New) The method of claim 30, wherein said microbial cell further comprises [further comprising] a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

61. (New) The method of claim 60 wherein the system further [comprising] comprises an expression gene wherein the expression gene encodes an antigen.

62. (New) The method of claim 61 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

63. (New) The method of claim 60 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,

wherein the cell is a member of the *Enterobacteriaceae*.

64. (New) The cell of claim 51 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

65. (New) The method of claim 46 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab* gene, or a *pls* gene.

66. (New) An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, wherein said essential gene is native to the cell, and wherein said essential gene is inactivated in the cell;

(b) a copy of said essential gene, wherein said copy is introduced into the cell, and wherein said copy is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(c) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

67. (New) The cell of claim 66 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

68. (New) The cell of claim 66 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of the *Enterobacteriaceae*.

69. (New) The cell of claim 66 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

70. (New) The cell of claim 69 wherein the vector has two lethal genes.

71. (New) The cell of claim 66 wherein expression of the essential gene is regulated by the expression product of a regulatory gene.

72. (New) The cell of claim 71 wherein the expression product of the regulatory gene inhibits expression of the essential gene and wherein said expression product is expressed or active only in the non-permissive environment.

73. (New) The cell of claim 69 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

74. (New) The cell of claim 66 further comprising an expression gene wherein the expression gene encodes a desired expression product.



75. (New) The cell of claim 66 [for use as a vaccine], wherein the cell is viable when in an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C, wherein the cell is a member of *Enterobacteriaceae*.

76. (New) The cell of claim 75 further comprising an expression gene wherein the expression gene encodes a desired expression product.

77. (New) A method of making a cell strain with environmentally limited viability comprising

(a) inactivating an essential gene in a cell, wherein expression of the essential gene in the cell is essential to the viability of the cell, and wherein said essential gene is native to the cell; and

(b) stably introducing into the cell

(i) a copy of said essential gene, wherein said copy is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(ii) a lethal gene, wherein expression of the lethal gene is lethal to the cell and wherein the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

78. (New) The method of claim 77 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

79. (New) The method of claim 77 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of *Enterobacteriaceae*.

80. (New) A method of inducing immunoprotection in a warm-blooded animal comprising  
administering to the animal [a vaccine comprising] a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, wherein said essential gene is native to the cell, and wherein said essential gene is inactivated in the cell;

(b) a copy of said essential gene, wherein said copy is introduced into the cell, and wherein said copy is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(c) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal, wherein the cell is a member of the *Enterobacteriaceae*.

81. (New) The method of claim 80 wherein the system further [comprising] comprises an expression gene wherein the expression gene encodes an antigen.

82. (New) The method of claim 81 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

83. (New) The method of claim 80 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,

wherein the cell is a member of the *Enterobacteriaceae*.

84. (New) The cell of claim 51 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

85. (New) The method of claim 66 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab*, gene, or a *pls* gene.